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(71) Applicant *(for all designated States except US)*: BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DEWITT, Douglas [–/US]; Kemah, TX (US). KRAMER, George, C. [–/US]; Tiki Island, TX (US). POLI DE FIGUEIREDO, Luiz, F. [–/US]; Galveston, TX (US). MATHRU, Mali [–/US]; Galveston, TX (US). PROUGH, Donald, S. [–/US]; Galveston, TX (US).

(74) Agent: KITCHELL, Barbara, S.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US).

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(54) Title: HYPERTONIC ARGININE COMPOSITIONS AND METHODS

(57) Abstract

The present invention concerns hypertonic formulations that areuseful to treat hemorrhage and trauma, and particularly trauma of the central nervous system, brain and spinal cord and circulatory shock. Also disclosed is a method of effectively treating or preventing the pulmonary or systemic hypertension that may occur with hemoglobin infusions. Such hypertonic formulations include L-arginine in various hypertonic aqueous formulations that may also include an oxygen carrier.

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DESCRIPTION

HYPERTONIC ARGININE COMPOSITIONS AND METHODS

1.0 BACKGROUND OF THE INVENTION

1.1 Field of the Invention

The present invention relates generally to the field of medical treatment. More particularly, it relates to improved hyperosmotic compositions for the treatment of hypovolemia, circulatory shock, traumatic brain injury (TBI), and hypoperfusion of the brain. Some of the hyperosmotic compositions include hemoglobin or various blood substitutes and others are hyperosmotic compositions without hemoglobin. The invention also concerns methods for prevention and treatment to reduce pulmonary and systemic vasoconstriction associated with infusion of hemoglobin solutions.

1.2 Description of Related Art

Trauma is the leading cause of death in young Americans between the ages of 1-44. TBI accounts for over 30% of all injury deaths in the United States and results in significant disability in 80,000 victims annually. Hypotension is the single most important secondary factor in increasing morbidity and mortality after TBI (Miller, 1981). The most common cause of hypotension is the hemorrhagic and circulatory shock that usually accompanies traumatic head injury.

Hemorrhage, hypovolemia, circulatory shock and trauma are common life threatening medical emergencies resulting from accidents, crime, terrorism, and war. Reduced blood volume lowers the blood pressure and cardiac output and oxygen delivery to critical organs in turn causing ischemia, organ dysfunction, and death. The brain is sensitive to ischemia and becomes particularly so when TBI accompanies hemorrhagic shock.

The brain's metabolism can only tolerate low cerebral blood flow (CBF) and inadequate cerebral oxygen delivery (CDO_2) for short time periods. TBI releases neuroexcitatory amines which increase the oxygen needs of the brain, while tissue

swelling and intracranial hemorrhage increase the intracranial pressure (ICP) which reduces CBF. Hypotension or reduced mean arterial pressure (MAP) further reduces brain CBF to dangerously low levels and explains why hypotension on hospital admission nearly doubles the mortality rate and incidents of vegetative state of TBI patients (Miller, 1981). A goal of shock resuscitation therapy is to improve the CBF and oxygen delivery to the brain and other tissues. Ideal therapy, yet to bc achieved, for TBI would be to lower the ICP, selectively vasodilate the brain, and correct and prevent hypotension and hemorrhagic shock.

Other clinical situations where brain damage can occur from reduced or arrested CBF include stroke, cerebral hemorrhage, hypothermia, dehydration, heat stroke, drowning and events occurring during and after cardiopulmonary bypass.

One approach to resuscitation is to use whole blood or oxygen carrying hemoglobin solutions. Although blood has several physiologic functions, the most basic and essential function is achieved by the red cells, which provide a continuous supply of oxygen to organs and tissues.

The important function of hemoglobin as an oxygen carrier emphasizes the need for red blood cell substitutes. Large quantities of blood are used daily in emergency care and surgery. Natural disasters and war casualties demand immediate availability of blood. Avoiding blood transfusion is a high priority because of the related potentially fatal side effects, including transmission of infectious diseases and transfusion reactions. In addition to the avoidance of transfusion related complications, advantages of blood substitutes include their role as a universal blood substitute for surgery and trauma without the need for cross-match interference. Other advantages would be long storageability, immediate availability, lack of viral or bacterial exposures, and moderate cost.

In addition to the oxygen-carrying capacity, several studies have shown pressor and perfusion properties of stroma-free hemoglobins when used in animal models of hemorrhagic shock or in whole blood exchange. The most striking cardiovascular effect observed with free hemoglobin is a profound vasoconstriction (Poli de Figueiredo and Mathru, 1997). When hemoglobin is free in solution, as with most blood substitutes it scavenges NO (nitrous oxide), thereby causing vasoconstriction. This scavenging ability appears to be a basic characteristic of the hemoglobin molecule.

NO is the final common pathway for many forms of vasodilation. Under normal physiologic conditions, where the hemoglobin is within the red cell, NO in the vessel wall is effective in maintaining vasodilatory tone and is removed as it dissolves into the plasma and ultimately attaches to hemoglobin (Poli de Figueiredo and Mathru, 1997). Other mechanisms that appear to contribute to the vasoconstrictor effect include: activation and release of endothelin, enhanced vascular sensitivity of the alpha-adrenergic receptors to circulating catecholamines, and release of platelet activator factor or related compounds (Poli de Figueiredo and Mathru, 1997).

Results from experimental work and initial human trials of free hemoglobin have been disappointing. The vasoconstriction effects of free hemoglobin solutions limit the effectiveness of these solutions for the treatment of shock. There remains therefore a need to treat or decrease the vasoconstriction caused by hemoglobin solutions and thus eliminating or lessening undesirable vasoconstriction effects.

2.0 SUMMARY OF THE INVENTION

The present invention relates to new hypertonic arginine formulations that effectively correct cerebral ischemia and hypoxia of the combined injury of TBI and hemorrhage. The disclosed resuscitation solutions, particularly when used in combination with hemoglobin or hemoglobin substitutes, would reduce or eliminate the vasoconstrictive properties of hemoglobin, diminishing the likelihood of vasoconstriction related complications and more effectively treating shock and trauma. These formulations thus address several significant problems in treating hemorrhagic shock, particularly where TBI is involved. The disclosed methods employing hypertonic arginine formulations are expected to have a significant societal and economic benefit in reducing the morbidity and mortality of TBI.

Although L-arginine was known to prevent hypoperfusion in the brain after moderate TBI when blood pressure is normal, the beneficial effect of hypertonic L-arginine formations on hemorrhagic hypotension or TBI and hemorrhage was unexpected. In fact, conventional wisdom in view of the state of knowledge concerning the effects of L-arginine would have predicted lower blood pressure by producing more vasodilation

through greater NO release. Use of L-arginine therefore would seem to be contra-indicated in treatment of hypotensive shock.

The invention in an important aspect relates to methods of treating hypovolemic shock and particularly to those types of shock that are associated with multifocal trauma such as where traumatic brain injury (TBI) is involved. L-arginine formulations in hypertonic media have been shown to have a surprising effect on brain blood flow by increasing brain blood flow while at the same time maintaining much lower intracranial pressure than lactated Ringers, a commonly used solution for increasing blood volume, or hypertonic saline. In a typical demonstration of this effect, a NaCl hypertonic solution containing L-arginine was used. This solution had an osmolarity of about 2,400 mOsm/L and on intravenous infusion delivered a dose of arginine in excess of 50 mg/kg in an infused volume of 4-6 ml/kg.

Of course, one is not limited to 2,400 mOsm/L solutions and suitable osmolalities may include from about 1000 mOsm/L up to about 5,000 with 1,500 to 3,000 being particularly preferred. Hypertonic solutions are not limited to ionic NaCl solutes and may include NaAcetate, Mannitol or other physiologically acceptable salts, carbohydrates or amino acids.

Certain advantages may be achieved by adding other components to the hypertonic L-arginine formulations. These may include ionic or nonionic species such as ATP-MgCl, fructose diphosphate or dichloroacetate.

It is also advantageous to add hyperoncotic colloids; for example, dextran, hetastarch, proteins or peptides, hemoglobin or hemoglobin substitutes. With respect to hemoglobins, several are known and available, including cell free human or animal hemoglobin, cross-linked hemoglobin, acellular alpha or beta chain cross-linked hemoglobin, recombinant hemoglobin, polymerized hemoglobin, PEG conjugated hemoglobin, bovine hemoglobin, hemoglobin conjugated with dextrans and liposome-encapsulated hemoglobin.

Some of these compounds act as plasma expanders and are contemplated to be most effective in these formulations at concentrations of about at least 5 g/100 ml.

It is evident that in most situations, the disclosed solutions will be employed in emergency situations, whether in a hospital environment or in the field as in a battlefield

situation. Administration will most conveniently be by intravenous or intraosseous injection such that L-arginine is delivered in hypertonic media at a dose of at least 50 mg/kg in an infused volume of at least 3 ml/kg and preferably higher; for example, at 4-6 ml/kg.

A preferred embodiment is a hypertonic solution of L-arginine that includes hemoglobin or hemoglobin substitute. The animal models illustrated here show that hypertonic L-arginine solutions significantly improve CBF and normalize cardiac output while reducing vasoconstriction in systemic and pulmonary circulation. These beneficial effects may be obtained by combining the hypertonic L-arginine formulations with hemoglobin, or alternatively, infusing separately with a hemoglobin or hemoglobin substitute.

While some adjustment may be necessary in determining optimum amounts of L-arginine in the disclosed formulations, it is believed that preferred effective concentrations are in the range of about 0.3 to about 7.5 g/100 ml. Where NaCl is used as the hypertonic medium, preferred ranges are about 6 to about 8 g/100 ml. As mentioned, osmolality of the solutions are generally in the 1,000-2,400 mOsm range; however, this is not to say that ranges from about 800 to about 5000 mOsm/L would not also be beneficial in certain applications. A convenient method to adjust osmolarity is to add or remove water.

3.0 BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A. shows changes in CBF of anesthetized rats subjected to TBI and hemorrhage after a 6 ml/kg treatment with ~2400 mOsm Arginine-NaCl solution made up of 7.28 g/100 ml NaCl and 1.67 g L-arginine, or ~2400 mOsm/L NaCl solution made up of 7.5 g/100 ml NaCl or an equal solute dose (48 ml/kg) of isotonic lactated Ringer's. The later solution is the current large volume isotonic standard of care for treating hemorrhage and brain injury.

FIG. 1B. shows the intracranial pressure after administration of the solutions described in FIG. 1A.

FIG. 2A. shows the resuscitative effects of 6 ml/kg of a ~2400 mOsm hypertonic arginine solution containing 7.28 g/100 ml NaCl and 1.67 g L-arginine (HArg) and a second experiment with HArg mixed with 10% alpha alpha free hemoglobin (HArg-Hb) as measured by changes in arterial blood pressure in anesthetized hemorrhaged rats subjected to TBI.

FIG. 2B. shows the changes in brain blood flow in rats administered the solutions described in FIG. 2A.

FIG. 3A. shows the effects of resuscitation on arterial pressure with 2 ml/kg of 10 g/100 ml of alpha alpha hemoglobin infused into conscious sheep after a 65 minute hemorrhage of 1300 ml. Ten minutes after the hemoglobin infusion the effects of 4 ml/kg of ~ 2400 mOsm or exactly 7.16 g/100 ml NaCl and 2.5 g/100 ml of L-arginine are shown.

FIG. 3B shows the effects of resuscitation on cardiac output of the sheep after administration of the solutions described in FIG. 3A.

FIG. 4A shows the calculated vascular resistance of the systemic circulation (mean arterial pressure divided by cardiac output) of the same sheep in FIGs. 3A and 3B during baseline, hemorrhage, 10 minutes after hemoglobin infusion and 10 minutes after L-arginine infusion.

FIG. 4B. shows the pulmonary vascular resistance (the pulmonary artery pressure and pulmonary wedge pressure difference divided by cardiac output) of the sheep administered the solutions described in FIG. 3A.

4.0 DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

4.1 Effect of Hemoglobin on Vasoregulation

A profound impact on vasoregulation of all circulatory beds is expected after infusion of hemoglobin solutions. Recent studies have demonstrated a marked increase in systemic vascular resistance, following the infusion of alpha alpha hemoglobin for small

volume resuscitation of hemorrhaged pigs (Poli de Figueiredo, 1997b). This rise in vascular resistance is the main mechanism for the near normalization of arterial blood pressure, as no increase was observed in cardiac output. Furthermore, a severe, and acute three-fold increase in pulmonary vascular resistance can be demonstrated, leading to pulmonary hypertension a cause of right ventricular failure. In hemorrhaged animals this pulmonary vasoconstriction may lead to overall hemodynamic instability and a further reduction in cardiac output below that due to hemorrhage alone (Poli de Figueiredo, 1997a).

4.2 Hyperosmotic Crystalloid/Hyperoncotic Colloid Formulations:

One approach to resuscitation without hemoglobin or similar oxygen carriers has been to infuse a small volume of concentrated hyperosmotic solution. Animal research and clinical testing of ~2400 mOsm, 7.5% hypertonic saline 6% dextran (HSD) has shown efficacy and safety, (Kramer, 1997; Wade, 1997b). Infusion of HSD in hemorrhaged animals replaces blood loss by using hypertonic saline-(HS) crystalloid to osmotically mobilize cellular water and the colloid-dextran to osmotically hold the water in the circulation, (Kramer, 1997). A solution of 7.5% hypertonic saline alone only transiently improves cardiovascular function, while the addition of 6% dextran sustains it.

Small volume hypertonic saline solutions of 7.2 to 7.5% NaCl are often described as 2400 mOsm NaCl. The most commonly used 7.5% NaCl solution is actually a total of 2,557 mMol of Na and Cl molecules per liter of solution.

However, HSD has limitations, including:

1) HSD increases blood pressure and lowers ICP, but only transiently improves brain CBF;

2) NaCl and dextran are relatively inert and devoid of specific pharmacologic effects that improve brain blood flow; and

3) As with all acellular intravenous fluids, HSD dilutes the red cell content and decreases the blood's oxygen content.

Replacing the HS with a solute with similar osmotic properties, but also containing beneficial pharmacologic properties and replacing the dextran with an oxygen carrying colloid has theoretical benefit. Recent experiments associated with this patent suggest that

we have discovered a far more effective hyperosmotic formulation for the treatment of TBI and hypotension.

4.3 Hyperosmotic Oxygen Carriers

The present invention has approached the resuscitation problem by combining a hyperosmotic solution with an oxygen carrier in one embodiment. A combined solution that expands plasma volume, increases cardiac output, increases CBF and blood oxygen content significantly improves cerebral oxygen delivery and is expected to impact survival and functional outcome of trauma patients.

Hypertonic oxygen carriers have been described in several publications and patents including hyperosmotic crystalloid-hyperoncotic colloid combinations such as, ~ 2400 mOsm NaCl- combined with protein such as hyperoncotic hemoglobin, (Kramer and Holcroft 1985); hypertonic solutions mixed with oxygen carrying perfluorocarbons or hemoglobins, (Runge 1989); hypertonic saline combined with liposome encapsulated hemoglobin (Rabinovici, 1993); and specific hypertonic sodium acetate chloride vasodilatory formulations combined with hemoglobin, (Rocha e Silva, Velasco and Kramer 1993; and Rocha e Silva, Velasco and Kramer and Wade, 1995). The hemoglobin molecule is similar in size to dextran, but carries oxygen. In solution, hemoglobin concentrations of 5 g/100 ml and above produces a hyperoncotic formulation.

A key physiologic role of the hyperosmotic crystalloid is to rapidly expand plasma volume due to osmotic induced movement of cellular water into the circulation, while a key role for the hyperoncotic macromolecular colloid is to hold that water in the circulation, (Kramer/Holcroft, 1985). A ~ 2400 mOsm/L solution has been shown to be a highly effective concentration for the hyperosmotic crystalloid component, (Kramer, 1997). Thus the inventors reasoned that hypertonic acetate hemoglobin would be desirable because of the strong vasodilatory properties of the acetate which can oppose the vasoconstriction of hemoglobin (Rocha e Silva, Velasco, Kramer and Wade, 1995). Additionally, it was known that such solutions combine the cardiovascular attributes of hyperosmotic-hyperoncotic solutions (rapid volume expansion, vasodilation, reduced afterload, increased contractility, reduction of cellular edema) with the oxygen carrying augmentation of hemoglobin.

However, the inventors were also aware that these previously described hypertonic oxygen carrying solutions generally make use of relatively inert NaCl or sugar solutions as their osmotic agent and are devoid of any properties that selectively improved CBF. (Kramer and Holcroft 1985; Rabinovici, 1993; Runge, 1994).

4.4 Previous work on L-arginine and Hypertonic Saline in TBI

Recent studies involving CBF measurements within a few hours of TBI have shown that nearly one third of severely injured patients have inadequate CBF hypoperfusion and that patients with post-traumatic hypoperfusion have a poorer prognosis than patients with higher CBF levels (Bouma, 1991 and 1992). Ischemic damage is present in a majority of trauma patients at autopsy, indicating that reduced CBF contributes to the pathophysiology of TBI (Graham, 1978). These results suggest that cerebral hypoperfusion, likely occurs very soon after injury and contributes significantly to the mortality and morbidity of TBI.

The effects of L-arginine in conjunction with hemoglobin have been studied, but L-arginine was reported to negate the beneficial resuscitative effects of hemoglobin (Sharma, 1995). Sharma et al, suggested that NO inhibition was required for the full beneficial effects of hemoglobin to be apparent. Isotonic arginine has been shown to reduce some of the vasoconstriction of hemoglobin. Rats infused with diaspirin alpha alpha cross-linked hemoglobin exhibited increased arterial blood pressure, which was returned to pre-hemoglobin levels by a 600 mg/kg dose of L-arginine (Katsuyama, 1994).

4.5 Pharmaceutical Formulations

The disclosed compositions may be used to effectively treat circulatory shock associated with decreased brain blood as occurs with traumatic head injury. Hemorrhagic shock is a common form of circulatory shock in which half or more of estimated blood volume (6% of body weight) is lost. For example, in a 70Kg man over 2 liters of blood loss can result in lowered blood pressure and reduced blood flow to all organs including the brain. "Standard of care" treatment as set forth by the American College of Surgeons is to restore blood volume with large volume of isotonic crystalloid such as lactated

Ringers or normal (0.9%) saline. Typically, resuscitative volumes equal to 3 times hemorrhaged blood volume are needed or six liters in this example.

In an important aspect of the inventions arginine/sodium chloride solutions are used as initial or early treatment of shock and traumatic brain injury in small volume doses of about 4-6 ml/Kg or about 250ml to 500ml in this example.

The hypertonic L-arginine compositions may also be administered parenterally, for example by intravenous infusion. Solutions of the free base or pharmacologically acceptable salts can be prepared in water.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of pyrogen free, sterile manufacture and storage.

Sterile infusible solutions are prepared by incorporating the L-arginine in the required amount in the appropriate hypertonic aqueous solvent with various of the other enumerated below, as required, followed by filtered sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The phrase "pharmaceutically and physiologically acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains salts, proteins, carbohydrates or amino acids as active ingredients are well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions; solid forms suitable for solution in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

The composition can be formulated in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the L-arginine) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as,

for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions and the like.

Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

5.0 EXAMPLES

5.1 Example 1

This example illustrates the effect of L-arginine in a rat injury model. It has been shown that CBF decreases significantly within 15 minutes after experimental TBI in rats (Yuan, 1988). Thus, experimental injury models in rats are well-suited for studying the mechanisms that contribute to post-traumatic cerebral hypoperfusion.

In order to determine whether post-traumatic hypoperfusion could be prevented, rats were treated after moderate TBI with L-arginine (100 mg/kg, i.v., 5 min post-TBI) (DeWitt, 1997). NO synthase, an enzyme in brain and blood vessels, converts L-arginine to NO, a potent vasodilator. L-arginine completely prevented post-traumatic hypoperfusion in rats with normal blood pressure after moderate experimental TBI (DeWitt, 1997). D-arginine, a stereoisomer which is not a substrate for NO synthase, had no effect on post-traumatic cerebral hypoperfusion.

5.2 Example 2

This example illustrates the beneficial effect of hyperosmotic arginine solutions in treating hemorrhagic shock in an animal model. Standard of care treatments of TBI and hemorrhage with large volume isotonic therapy has often been shown to inadequately restore arterial pressure, elevate ICP and cause sustained reductions in CBF.

This experiment shows that a small volume IV infusion of ~ 2400 mOsm Arginine NaCl solution (7.28 g NaCl and 1.67 g/100 ml L-arginine) lowers ICP and improves CBF better than small volume 2,400 mOsm NaCl or large volume of isotonic "standard of care" resuscitation.

Three rats per group were anesthetized with isoflurane and had arterial and venous catheters placed for bleeding, infusion, and pressure monitoring. A laser Doppler flow probe was placed above the dura to allow monitoring of CBF and a intracranial pressure catheter was placed to allow measurement of intracranial pressure. After collecting baseline data, a moderate (1.5 atmosphere) percussion injury was administered through a fluid column connected by catheter which had been cemented through the skull to the dura. Five minutes later blood pressure was reduced to 60 mmHg for 30 minutes by bleeding. The hemorrhage and brain injury reduced CBF 30 to 70% from its baseline levels.

FIG. 1A shows elevated ICP after lactated Ringer's treatment and lower ICP with hypertonic saline and particularly with hypertonic arginine. FIG. 1B also shows higher CBF after 6 ml/kg hypertonic arginine infusion compared to 6 ml/kg hypertonic saline or 48 ml/kg large volume isotonic lactated Ringer's.

5.3 Example 3

Using the same animal model described in Example 1, a hypertonic arginine alpha cross-linked hemoglobin oxygen carrying colloid solution was infused through a venous catheter to treat hemorrhagic shock in three TBI rats. The solution of the hyperosmotic crystalloid was 7.28 g/100ml NaCl and 1.67g/100ml L-arginine, while the hemoglobin was 10g/100ml free human hemoglobin modified by cross-linking between the alpha subunits with bis-(3,5-dibromosalicyl) fumarate (alpha alpha Hb) and prepared according to previously published methods, (Winslow, 1992). The small volume solutions were infused evenly over 6 minutes.

FIGs. 2A and 2B show higher CBF and blood pressure after TBI and hemorrhage when treated with a 6 ml/kg intravenous infusion of hypertonic ~2,400 mOsm arginine hemoglobin solution compared to hypertonic arginine alone. Both of the hypertonic

arginine solutions were more effective than equal volume of hypertonic saline or a larger volume of lactated Ringer's, FIGs 1A and 1B.

5.4 Example 4

This example shows that hypertonic arginine solutions may be used to treat the systemic and pulmonary vasoconstriction of hemoglobin infusions and improve the resuscitative properties of hemoglobin containing blood substitutes. This illustrates how a hyperosmotic arginine solution could be used to treat the deleterious vasoconstriction of hemoglobin solutions or suggests that a premixed or concurrently delivered dose of hyperosmotic arginine with hemoglobin would be a most effect resuscitation solution.

A 44 kg sheep was subjected to a 1300 ml hemorrhage over 65 minutes which reduced cardiac output and blood pressure, FIGs. 3A and 3B. A two minute intravenous infusion with a small volume of 10% alpha alpha cross-linked hemoglobin blood substitute increased blood pressure to normal, but did not improve cardiac output. The mechanism of the increased blood pressure was vasoconstriction evident in the systemic and pulmonary circulation.

A subsequent infusion of 4 ml/kg of a ~ 2400 mOsm/L hypertonic arginine solution made up of 7.16 g/100 ml of NaCl and 2.5 g/100 ml of L-arginine had little effect on blood pressure, but improved and returned cardiac output towards normal, FIGs. 3A and 3B and reduced the vasoconstriction in systemic and pulmonary circulation, FIGs. 4A and 4B.

Such improved cardiac output would be expected to result in superior blood flow and oxygen delivery to most organs.

5.5 Example 5

This example shows several hypertonic arginine formulations that will be useful for treatment of trauma and shock. These are illustrated by the following:

A ~2400 mOsm hypertonic NaCl and L-arginine mixture for use alone or combined with various hyperoncotic colloids such as dextran, hespan, and other macromolecules such as hemoglobin. Such solutions may be used to treat trauma, hemorrhage, TBI, dehydration, heat stress, spinal cord injury, subarachnoid hemorrhage,

in the vasoconstriction of other conditions such as migraine, and in others conditions of cerebral ischemia such as cardiac surgery.

A ~ 2400 mOsm/L concentration of a mixture of hypertonic crystalloid NaCl/Arginine is made from the following g/100 ml concentrations to deliver an effective dose in a 4 ml/kg infusion:

TABLE 1

Hyperosmotic Solutes g/100 ml		Arginine Dose
NaCl	Arginine	mg/Kg
6.47	7.50	300
7.16	2.50	100
7.33	1.25	50

Alternately, for a 6 ml/kg infusion, the ~2400 mOsm/L solution could be made from the following g/100 ml concentrations:

TABLE 2

Hyperosmotic Solutes g/100 ml		Arginine Dose
NaCl	Arginine	mg/Kg
6.81	5.00	300
7.28	1.67	100
7.39	0.83	50

Such hypertonic crystalloids are contemplated as particularly useful solutions when combined with a colloid or an oxygen carrier such as hemoglobin, fluorocarbon, or similar combination.

Combining the hypertonic crystalloid formulations of Table 1 or Table 2 with an oxygen carrying hemoglobin or fluorocarbon would provide an effective solution, particularly when the solution is also hyperoncotic due to a macromolecular colloid component as shown in Table 3. Free hemoglobin solutions at concentrations above 5% or 5g/100 ml are normally hyperoncotic. Hemoglobin encapsulated in liposomes or vesicles or fluorocarbon solutions do not generate significant oncotic pressure and would

benefit from the addition of a colloid to produce a hyperoncotic solution. The hyperosmotic NaCl-Arginine crystalloid formulations of Table 1 and 2 could be made-up as hyperoncotic colloids by adding the following concentrations of hemoglobin, dextran, hespan or other macromolecular plasma expanders.

TABLE 3

Hyperosmotic Crystalloid (~2400 mOsm/L)	Oxygen Carrier	Hyperoncotic Colloid (concentration in g/100 ml)
NaCl	free hemoglobin	hemoglobin (5-25)
NaCl-arginine	Liposome Encapsulated hemoglobin	dextran/hetastarch (5-25)
NaCl-arginine	fluorocarbon	dextran/hetastarch (5-25)

5.6 Example 6

Cerebral vasospasm, a severe narrowing of the cerebral arteries, is a major cause of mortality and morbidity in patients after subarachnoid hemorrhage. Expansion of intravascular volume is one of the standard methods of treatment of vasospasm (Heros and Zervas, 1983). Recent evidence indicates that L-arginine dilates cerebral arteries (Morikawa, et al., 1994) and reduces the vasoconstriction of experimental vasospasm due to subarachnoid hemorrhage (Kajita, et al., 1994). It is contemplated that treatment of patients after subarachnoid hemorrhage with hypertonic volume expanding fluids containing L-arginine could serve both vital functions of volume expansion along with the direct cerebral vasodilatory or anti-vasoconstrictor effects of L-arginine.

5.7 Example 7

Cardiac surgery, whether used to repair defective heart valves in children and adults or to repair clogged heart blood vessels, is associated with ischemic damage to the heart and brain in some patients (Roach, 1996; Tsui, 1996). Hypertonic saline-colloid solutions have been shown to have volume requirements, reduce edema and improve respiratory function, (Boldt, 1993). L-arginine has been reported to improve metabolic

recovery in ischemic myocardium (Carrier, 1996) and to improve CBF after deep hypothermic cardiac arrest (Tsui, 1996). Arginine-containing hypertonic solutions with or without hemoglobin could be used for fluid replacement during or after cardiac surgery in order to provide an additional level of protection to the heart and brain by improving blood flow and oxygen delivery.

All of the compositions, methods and/or apparatus disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions, methods and apparatus and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

6.0 REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. A method of treating cerebral ischemia secondary to conditions associated with shock, trauma, dehydration, heat stress, cardiac surgery or hypotension, comprising administering a hypertonic composition comprising L-arginine and a crystalloid selected from the group consisting of sodium chloride, sodium acetate, sodium bicarbonate and sodium lactate wherein the L-arginine is at least 9.8 g/100 ml.

2. The method of claim 1 further comprising administering Hb or a Hb substitution in combination with the hypertonic composition.

3. A method of treating hypovolemic shock and/or trauma comprising administering to a subject in need thereof a pharmaceutically acceptable hypertonic L-arginine composition having an osmolarity of at least 1000 mOsm/L.

4. The method of claim 3 wherein administration of the hypertonic arginine is effected prior to or concurrent with administration of a blood substitute.

5. A method of treating pulmonary hypertension and vasoconstriction associated with administration of cell-free or hemoglobin blood substitute, comprising administering to a subject previously treated with said blood substitute an amount of hypertonic L-arginine effective to reverse or restore pulmonary vascular tension to near normal levels.

6. A hypertonic composition comprising L-arginine and a crystalloid selected from the group consisting of sodium chloride, sodium acetate, sodium bicarbonate and sodium lactate wherein the L-arginine is at least 0.8 g/100ml.

7. The hypertonic composition of claim 6 wherein the composition osmolarity is at least 1,000 mOsm/L.

8. The hypertonic composition of claim 6 wherein the composition osmolarity is about 2,400 mOsm/L.

9. A hyperosmotic crystalloid composition comprising L-arginine, NaCl and hemoglobin having a composition osmolarity of about 2400 mOsm.

10. A hypertonic composition comprising L-arginine and a hyperoncotic colloid selected from a group consisting of dextran, hetastarch, protein, hemoglobin substitute and hemoglobin wherein said composition osmolarity is greater than 1,000 mOsm/L.

11. The hyperosmotic composition of claim 6 or 7 further comprising a plasma expander having a concentration of at least 5 g/100 ml.

12. The hypertonic composition of claim 10 wherein the hemoglobin substitute is selected from the group consisting of cell free hemoglobin, cross-linked hemoglobin, acellular alpha or beta chain cross-linked hemoglobin, polymerized hemoglobin, conjugated hemoglobin, recombinant hemoglobin, bovine hemoglobin, hemoglobin conjugated with dextrans and liposome-encapsulated hemoglobin.

13. The hypertonic composition of claim 6 wherein the L-arginine concentrations is from about 0.5 to about 7.5 g/ 100ml.

14. The hypertonic composition of claim 6 wherein the NaCl concentration is from about 6 g/100ml to about 8 g/100ml.

15. The hypertonic composition of claim 6 wherein the osmolarity of the hypertonic composition is about 800-5,000 mOsm/L.

16. The hyperosmotic composition of claim 10 wherein the L-arginine is from about 1.0 to about 2.5 g/100 ml.

figure 1A

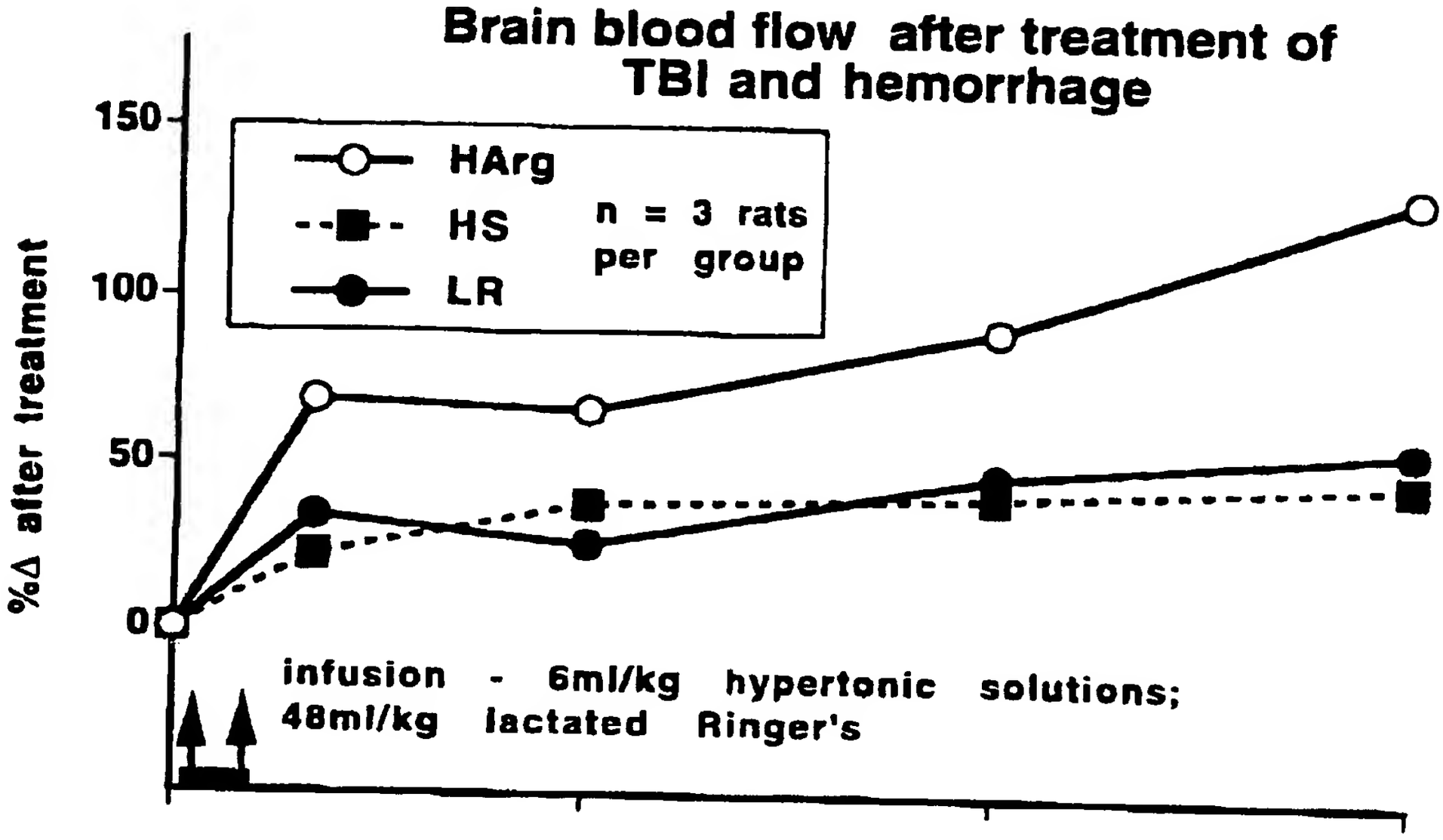


figure 1B

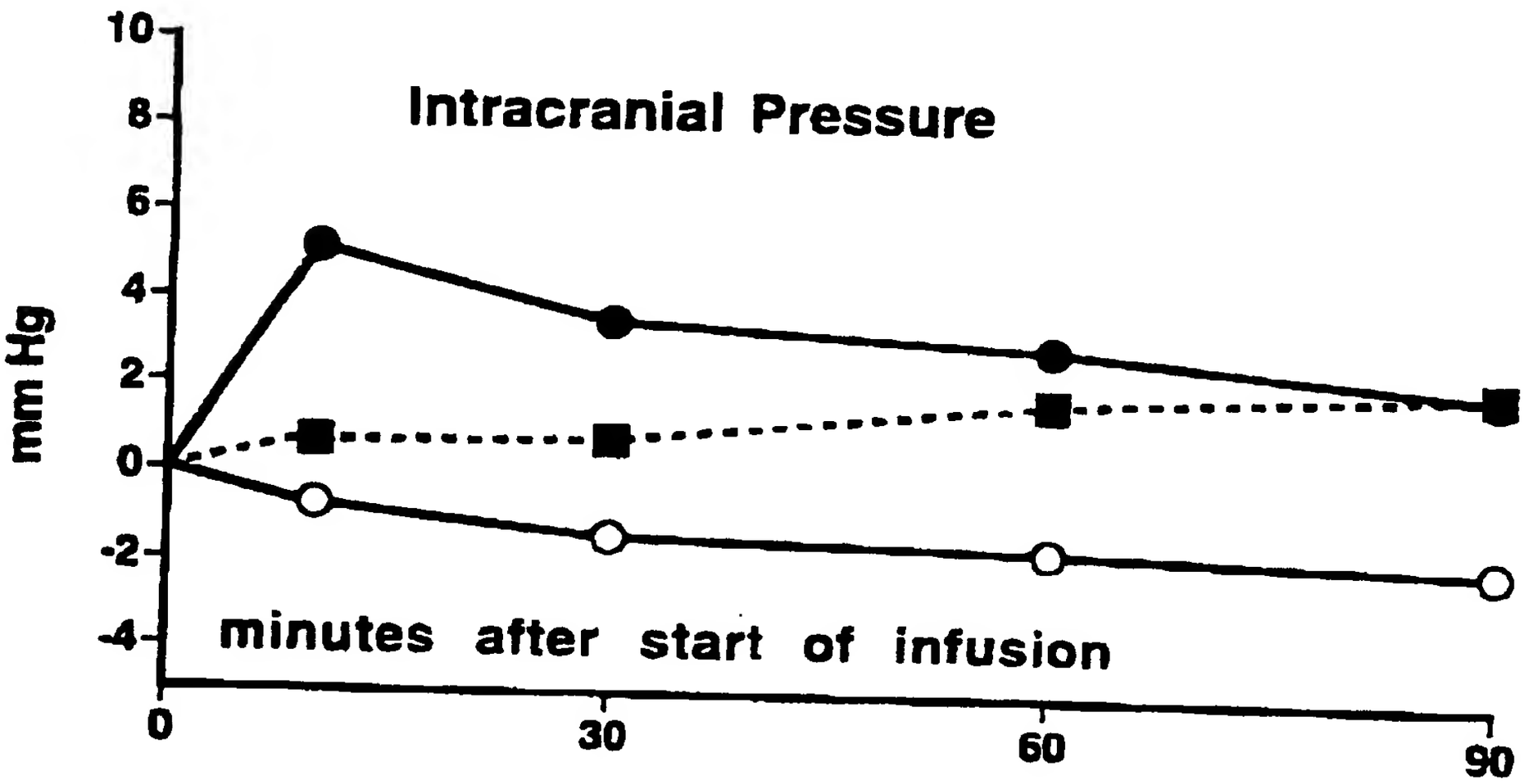


figure 2A

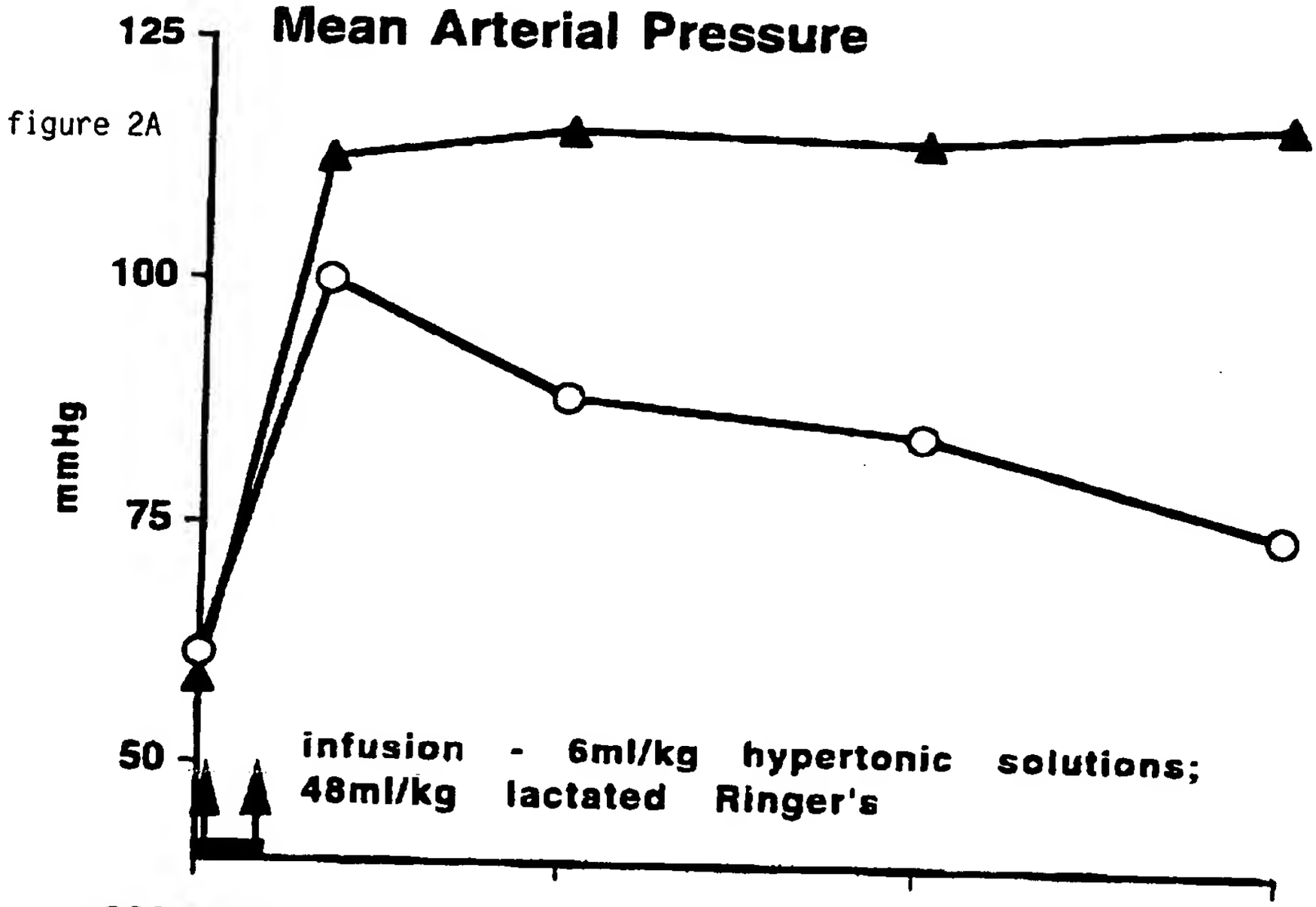


figure 2B

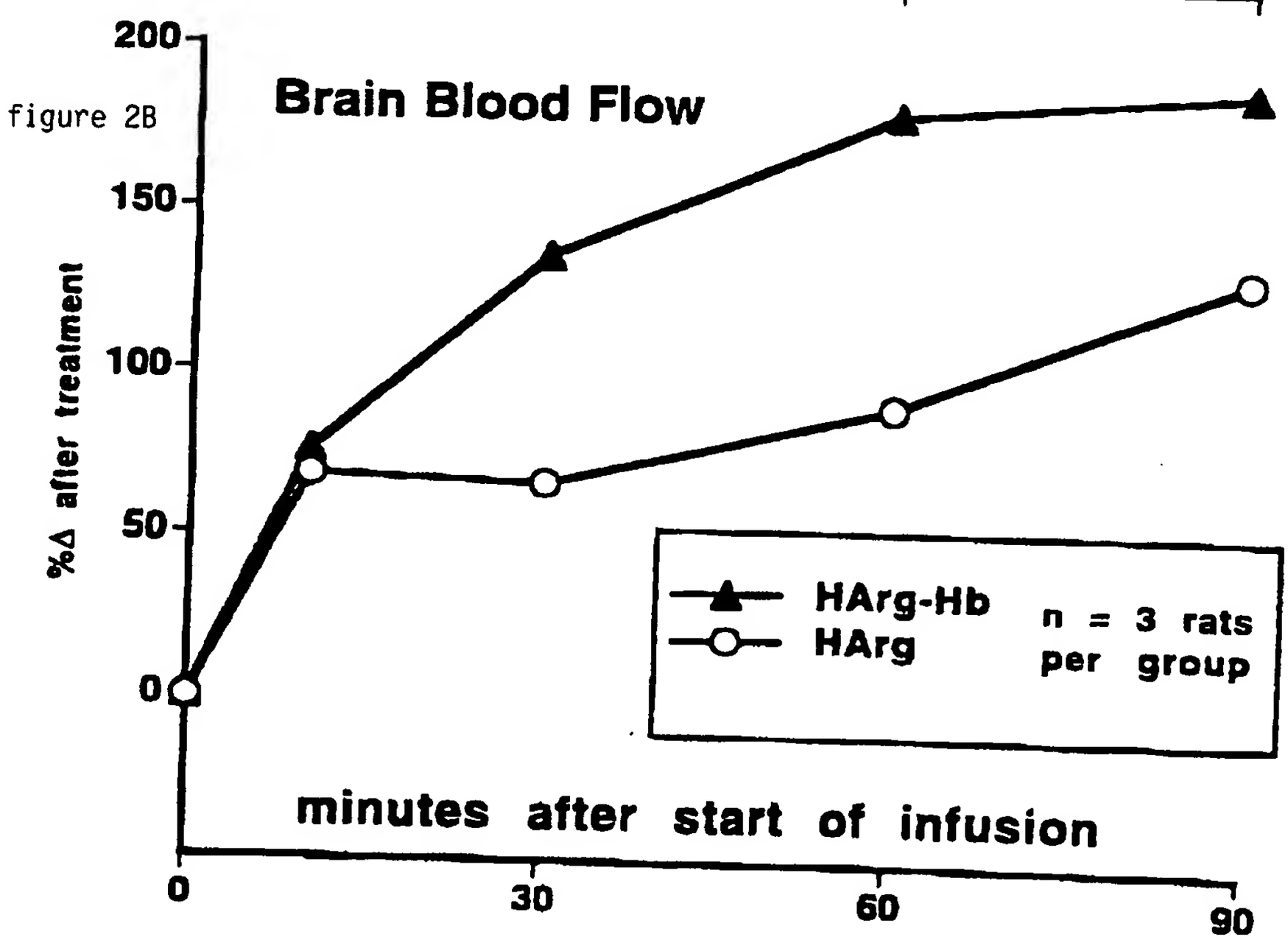


figure 3A

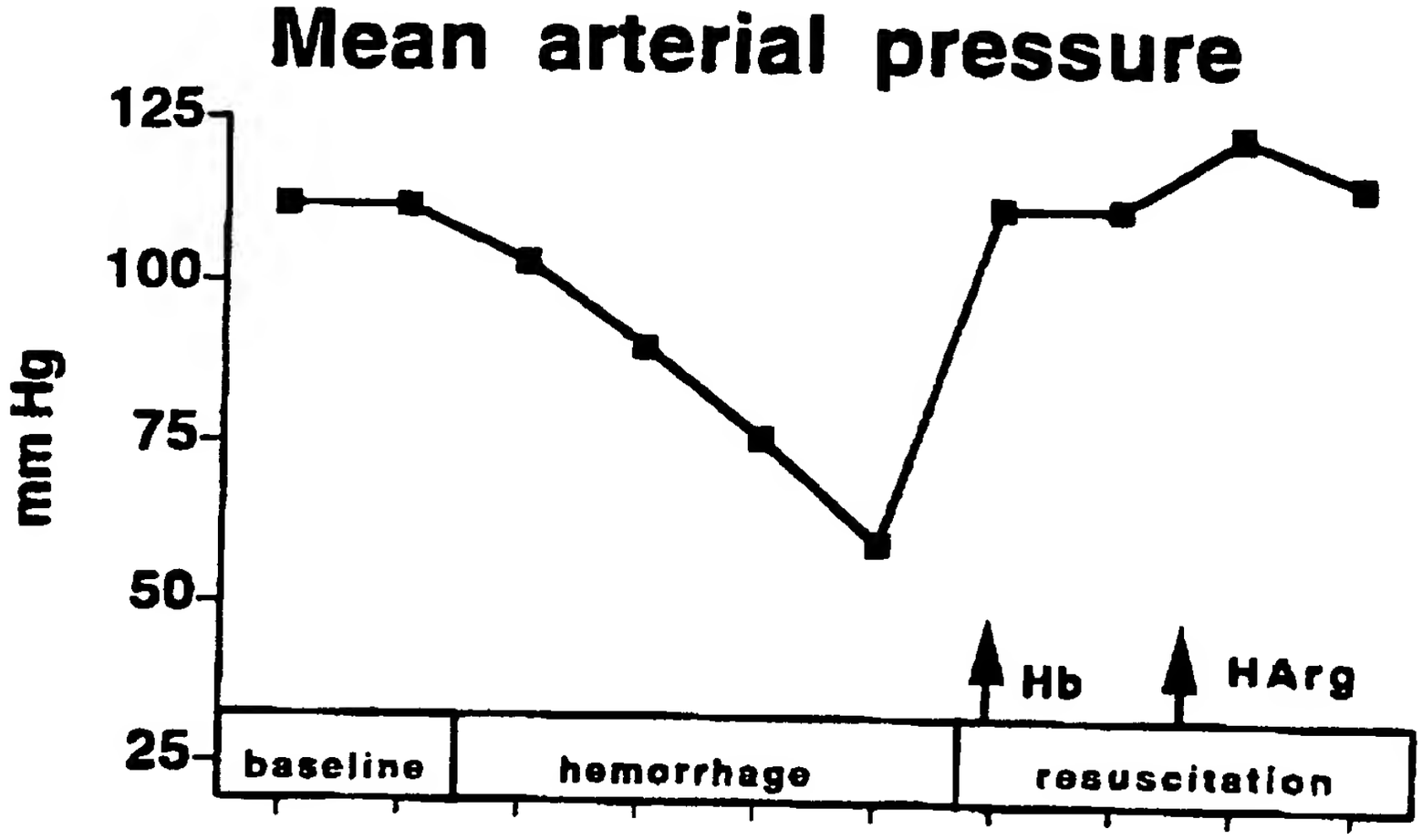


figure 3B

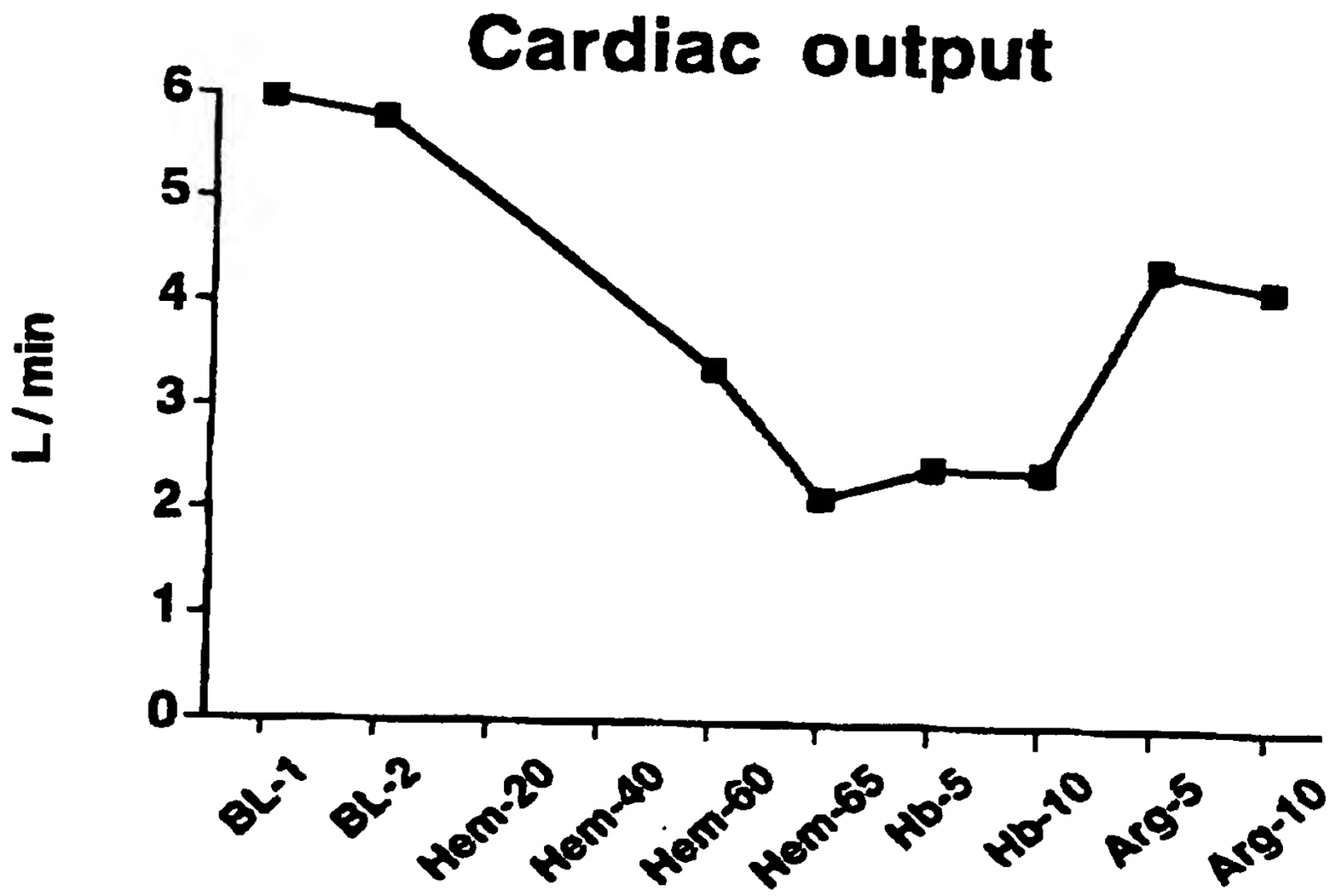


figure 4A

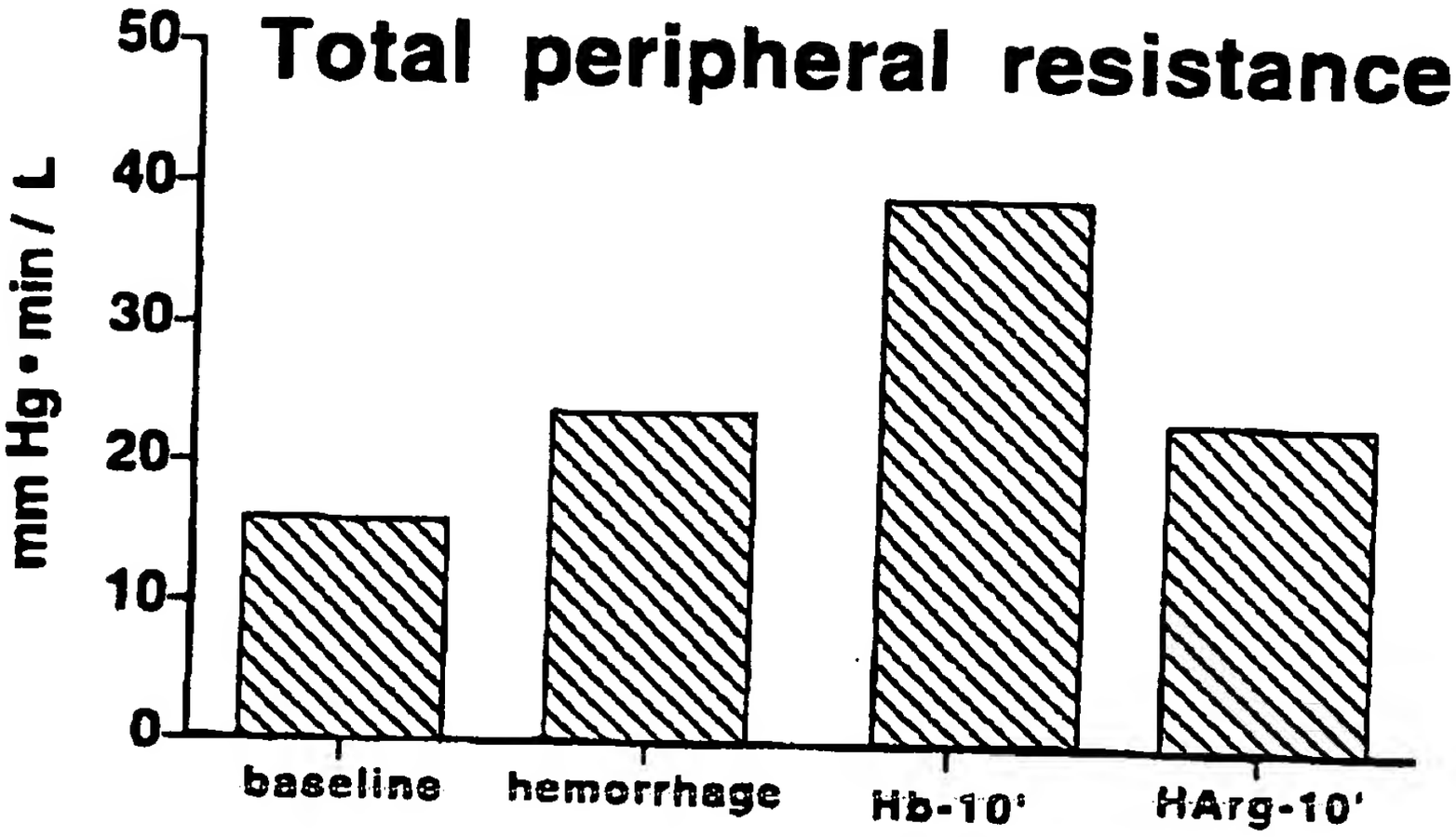
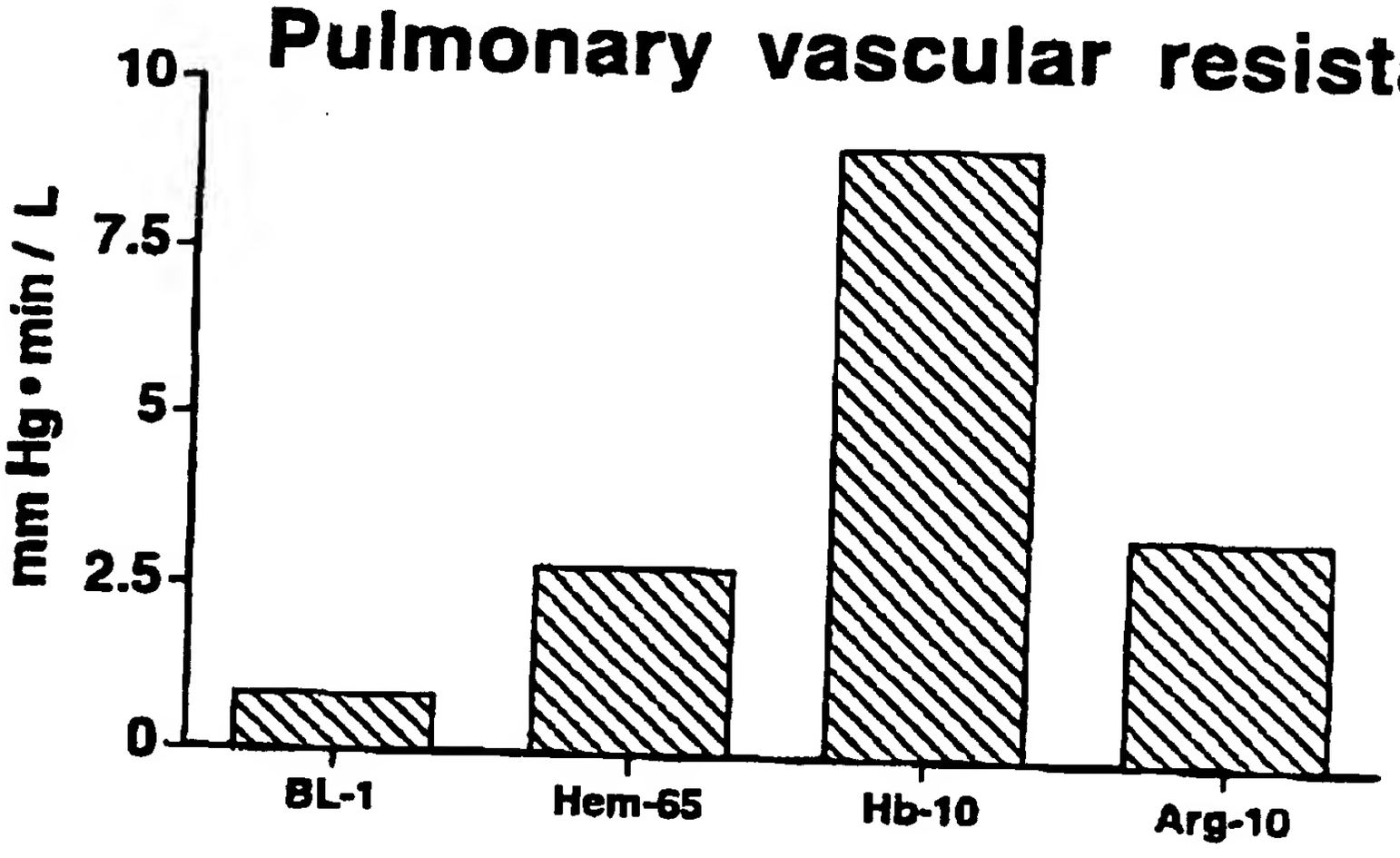


figure 4B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16203

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/195, 38/42; C07K 14/805
US CL :514/2, 557; 530/380; 562/553

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 557; 530/380; 562/553

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN ONLINE
search terms: arginine, cerebral ischemia, hemoglobin, hypertension

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,217,997 A (LEVERE ET AL) 08 June 1993 (08/06/93), see entire document.	1-16
Y	US 5,443,848 A (KRAMER ET AL) 22 August 1995 (22/09/95), see entire document.	1-16
Y	COLE et al. Focal Cerebral Ischemia in Rats Effects of Hypervolemic Hemodilution with Diaspirin Cross-linked Hemoglobin Versus Albumin on Brain Injury and Edema. Anesthesiology. February 1993, Vol. 78, No. 2, pages 335-342, see entire document.	2, 4-5, 9-10, 12, 16
Y	SHARMA et al. Role of NO mechanism in cardiovascular effects of diaspirin cross-linked hemoglobin in anesthetized rats. American Journal of Physiology. 1995, Vol. 269, No. 4, Pt. 2, pages H1379-H1388, see entire document.	2, 4-5, 9-10, 12, 16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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